

# High-affinity-receptor-mediated uptake and degradation of glucose-modified proteins: A potential mechanism for the removal of senescent macromolecules

(macrophage/nonenzymatic glycosylation/aging/endocytosis/advanced glycosylation end product)

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**ABSTRACT** Proteins that have been modified by long-term exposure to glucose accumulate advanced glycosylation end products (AGE) as a function of protein age. In these studies, we have characterized the interaction of AGE-protein with mouse peritoneal macrophages, using AGE-modified bovine serum albumin (AGE-BSA, prepared by incubation with glucose) as a probe. AGE-BSA was specifically bound to cells at 4°C and was taken up and degraded at 37°C; these processes were concentration dependent and saturable. Competition experiments with AGE-BSA, BSA incubated with phosphate-buffered saline rather than glucose, and yeast mannan demonstrated that macrophages specifically recognize AGE on proteins by a receptor that is completely distinct from the mannose/fucose receptor. Scatchard analysis of AGE-BSA binding data indicated that there are  $\approx 1.06 \times 10^5$  receptors per macrophage, with an affinity constant of  $1.75 \times 10^{-11}$  M. Specific binding of AGE-BSA to the macrophage receptor was competitively inhibited by BSA that had been chemically coupled to a synthetic analogue of the specific AGE, 2-(2-furoyl)-4(5)-(2-furanyl)-1H-imidazole (FFI-BSA). FFI-BSA was also taken up by macrophages in a concentration-dependent, saturable manner. Prior incubation of macrophages with AGE-BSA failed to influence the subsequent uptake and degradation of added AGE-BSA. Thus, the AGE receptor does not appear to be down-regulated by exposure to AGE-proteins. Results from these studies suggest that AGE could act *in vivo* as a specific signal for recognition and degradation of senescent macromolecules. Incomplete removal of AGE-proteins by macrophages may ultimately give rise to some of the physiologic changes that occur with normal aging.

As long-lived extracellular proteins such as collagen age, they are degraded and replaced at a constant slow rate throughout adult life (1). The mechanism by which these senescent macromolecules are specifically recognized and removed has not yet been elucidated. *In vivo*, nonenzymatic glycosylation products which accumulate on proteins as a function of protein age (2–4) could act as specific signals for recognition and degradation (5).

Nonenzymatic glycosylation begins with glucose attachment to protein amino groups. Initially, chemically reversible Schiff base and Amadori adducts form in proportion to glucose concentration. Equilibrium is reached after several weeks, however, and further accumulation of these early nonenzymatic glycosylation products does not continue (6, 7). Subsequently, a series of further reactions and rearrangements of the Amadori product slowly give rise to nonequilibrium advanced glycosylation end products (AGE). In contrast to the Amadori product, these adducts, once formed, are irreversible. Consequently, AGE continue to

accumulate indefinitely on long-lived proteins, such as collagen, lens crystallin, basement membrane, and myelin (8). The chemical structure of one AGE-protein adduct formed *in vitro* at physiologic pH and temperature has been determined (9). This yellow fluorescent compound, 2-(2-furoyl)-4(5)-(2-furanyl)-1H-imidazole (FFI) is a condensation product of two glucose molecules and two lysine  $\epsilon$ -NH<sub>2</sub> groups. *In vivo* formation of FFI has been demonstrated on several human proteins (8, 10) by use of a recently developed radioimmunoassay.

We have previously investigated the biological consequences of AGE formation on rat and human peripheral nerve myelin and reported that protein modification by these products promotes specific recognition and uptake by mouse macrophages (5, 11). Macrophage recognition and accumulation of myelin glycosylated *in vitro* increased with duration of incubation with sugar, and intracellular accumulation of myelin from diabetic patients was 2–3 times higher than for myelin from normal individuals of the same age. Competition experiments with *in vitro*-glycosylated myelin and albumin showed that macrophage recognition was specific for AGE (5). Proteins modified primarily with Amadori products were not recognized by mouse macrophages. These observations suggested that AGE formed *in vivo* might function as a specific signal for macrophage removal of senescent structural proteins.

We now have further characterized the interaction of AGE-proteins with macrophages, using AGE-modified bovine serum albumin (BSA) and BSA with chemically attached FFI as probes. Our results indicate the presence of a high-affinity receptor on macrophages that mediates the uptake and degradation of AGE-proteins.

## METHODS

**Preparation of AGE-BSA.** AGE-BSA and (P<sub>i</sub>/NaCl)BSA were prepared by incubating BSA in 0.15 M phosphate, pH 7.2/0.15 M NaCl (P<sub>i</sub>/NaCl) with or without 50 mM glucose, respectively, at 37°C for 3–4 weeks in the presence of 1.5 mM phenylmethylsulfonyl fluoride, 0.5 mM EDTA, penicillin (100 units/ml), and gentamycin (40 µg/ml). The amount of the stable but reversible Amadori product 1-amino-1-deoxyfructose present in these preparations was determined by reduction with NaBH<sub>4</sub>, followed by hydrolysis and boronic acid chromatography (5, 12). AGE-BSA samples averaged  $10,600 \pm 120$  cpm/µmol of total amino acid, whereas (P<sub>i</sub>/NaCl)BSA averaged only  $830 \pm 58$  cpm/µmol of total amino acid. The amount of the post-Amadori rearrangement product FFI was measured in the same albumin samples by use of a recently developed radioimmunoassay system

(10). The AGE-BSA samples averaged  $1750.0 \pm 275$  pmol of FFI/mg of BSA, whereas  $(P_i/NaCl)BSA$  samples averaged  $9.2 \pm 1.0$  pmol of FFI/mg of BSA.

**Preparation of FFI-Hexanoic Acid and FFI Linked to BSA (FFI-BSA).** The synthesis of FFI-hexanoic acid is described in detail elsewhere (9, 10). In brief, furylglyoxal hydrate (10 mmol) in dioxane/water (3:1, vol/vol) was treated with 6-aminohexanoic acid (15 mmol) and triethylamine (15 mmol) and stirred at 25°C for 1 hr. After the addition of concentrated aqueous ammonia, the mixture was diluted with 5%  $NaH_2PO_4$  and extracted with  $CH_2Cl_2$ , washed with brine, and filtered through activated carbon and  $MgSO_4$ . Approximately 1.0 g of crude product was purified by medium-pressure chromatography on silica gel, yielding FFI-hexanoic acid as straw-colored flakes (280 mg, 16.3% yield, m.p. 105–106°C). FFI-BSA was prepared by covalently coupling FFI-hexanoic acid to BSA with water-soluble carbodiimide (10). FFI-hexanoic acid (11.2 mg) was dissolved in 1.0 ml of distilled  $H_2O$  containing 10  $\mu$ l of triethylamine and incubated with BSA (20 mg). After addition of 1-cyclohexyl-3-[2-(4-morpholinyl)ethyl]carbodiimide (14 mg, Aldrich), the mixture was incubated at room temperature overnight and dialyzed vs. distilled water at 20°C for 48 hr. Spectroscopic analysis of the resultant clear yellow solution indicated a BSA/FFI molar ratio of 1:12 ( $\epsilon_{365}$  9664); RIA indicated a molar ratio of 1:10.4.

**Isolation of Mouse Peritoneal Macrophages.** Female NCS mice (25–30 g) were obtained from the Laboratory Animal Research Center facility of The Rockefeller University. Elicited macrophages were harvested in phosphate-buffer saline 6 days after the intraperitoneal injection of 3.0 ml of Brewer's thioglycolate broth (Difco) as described by Edelson and Cohn (13). The fluid from 15–20 mice ( $6.8 \times 10^6$  cells per mouse) was pooled and centrifuged at  $500 \times g$  for 10 min, washed once with 20 ml of Dulbecco's modified Eagle's medium (GIBCO), and resuspended in medium containing 10% fetal calf serum, penicillin (100 units/ml), and streptomycin (100  $\mu$ g/ml). After total cell counts and differential cell counts were performed, aliquots containing  $2 \times 10^6$  cells were seeded in Linbro plastic dishes (1.0  $\times$  3.5 cm) (Linbro) and incubated at 37°C in 5%  $CO_2$  for 2 hr. The monolayers then were washed three times with medium and each well received 1.0 ml of fresh medium containing 10% fetal calf serum and various amounts of the indicated ligands. Each well contained  $\approx 10^6$  cells, equivalent to  $\approx 160$   $\mu$ g of protein.

**Iodination of Proteins.** Portions of each protein sample were radioiodinated with carrier-free  $^{125}I$  (New England Nuclear) by the IODO-GEN method of Fraker and Speck (14). Samples were dialyzed against a 1000-fold excess of 0.01

M phosphate, pH 7/0.15 M NaCl until >95% of the radioactivity was trichloroacetic acid-precipitable and the samples were iodide free. Protein concentrations were determined by the method of Bradford (15). Specific activities varied from 250 to 950 cpm/ng of protein.

**Uptake, Degradation, and Binding Studies.** Macrophage uptake and degradation experiments were performed at 37°C as described (5). Macrophage accumulation of the different radiolabeled ligands [AGE-BSA,  $(P_i/NaCl)BSA$ , FFI-BSA] was evaluated by incubating various concentrations of each with cells for 4 hr. The cell monolayer then was washed five times and lysed by addition of 0.1 M NaOH. Cell-associated radioactivity was determined with a Packard TriCarb Scintillation counter and cellular protein was measured by the method of Bradford (15). For pretreatment experiments, cells were incubated with unlabeled AGE-BSA or FFI-BSA (250  $\mu$ g/ml) for 72 hr prior to determination of uptake. Degradation was determined by measuring trichloroacetic acid-soluble radioactivity in the aspirated media. Competition experiments were performed as described (5), using various amounts of  $^{125}I$ -labeled AGE-BSA and various concentrations of potentially competing unlabeled compounds [AGE-BSA,  $(P_i/NaCl)BSA$ , mannan, and FFI-BSA] at either 37°C or 4°C.

Binding experiments were carried out at 4°C. One hour after being seeded, the cells were washed three times and allowed to equilibrate at 4°C in fresh medium. Binding was initiated by aspirating the wash medium and adding 1.0 ml of the incubation medium, containing the radiolabeled material. Cells were agitated gently at 4°C for 2 hr. The radioligand-containing medium then was aspirated, and the cells were washed three times with 1.0 ml of fresh medium at 4°C. Cells were dissolved overnight in 1.0 ml of 0.1 M NaOH per well. Cell-associated radioactivity and cellular protein were determined as described above. Total binding was defined as the amount of labeled ligand bound under these conditions. Nonspecific binding was defined as the radioligand bound in the presence of a 100-fold molar excess of unlabeled ligand. Specific binding was calculated as the difference between total and nonspecific binding. All data points represent duplicate or triplicate values. Transformation of saturation binding data was performed according to Scatchard (16) in order to estimate receptor number and binding affinity constant.

## RESULTS

Incubation of BSA with glucose as described above resulted in formation of  $1750 \pm 275$  pmol of the advanced glycosyla-

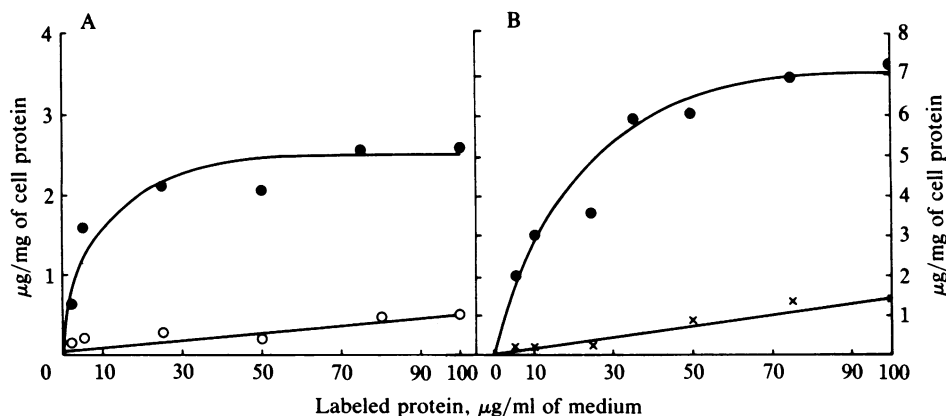


FIG. 1. Accumulation (A) and degradation (B) of  $^{125}I$ -labeled AGE-BSA ( $\bullet$ ) and  $^{125}I$ -labeled  $(P_i/NaCl)BSA$  ( $\circ$ ,  $\times$ ) by mouse peritoneal macrophages. After a 4-hr incubation at 37°C with medium containing the indicated concentration of ligand, the amount of radiolabeled protein in the cells (A) and the amount of trichloroacetic acid-soluble  $^{125}I$ -labeled material in the medium (B) were determined in duplicate.

tion end product FFI/mg of BSA. In contrast,  $(P_i/NaCl)BSA$  contained only  $9.2 \pm 1$  pmol of FFI/mg of BSA. When aliquots of AGE-BSA,  $(P_i/NaCl)BSA$ , and FFI-BSA were subjected to gel-filtration chromatography (Bio-Gel A-1.5 m, Bio-Rad) in 0.1 M potassium phosphate (pH 7.4) and to NaDodSO<sub>4</sub>/12% PAGE, no BSA dimers or high molecular weight aggregates were detected (data not shown).

When macrophages were incubated at 37°C for 4 hr with increasing concentrations of <sup>125</sup>I-labeled AGE-BSA, cell-associated radioactivity increased in a saturable fashion (Fig. 1A). At the same time, AGE-BSA degradation, measured as trichloroacetic acid-soluble material (Fig. 1B), also increased with concentration in a saturable fashion. Maximal values of both accumulation and degradation were reached at concentrations of 30–50 µg/ml. In contrast, only small amounts of <sup>125</sup>I-labeled  $(P_i/NaCl)BSA$  were taken up and degraded by the cells.

To assess the specificity of AGE-BSA uptake by the cells, we performed competition experiments using <sup>125</sup>I-labeled AGE-BSA or  $(P_i/NaCl)BSA$  (10 µg/ml) and various concentrations of unlabeled AGE-BSA or  $(P_i/NaCl)BSA$ , respectively (Fig. 2). AGE-BSA effectively competed with labeled AGE-BSA, suppressing uptake to <10% of the control value (0.460 µg/mg of cell protein) (Fig. 2A). AGE-BSA also competed with labeled  $(P_i/NaCl)BSA$  (Fig. 2B), suppressing uptake to ≈24% of control (0.045 µg/mg of cell protein). This result suggests that  $(P_i/NaCl)BSA$  contains small amounts of AGE. However,  $(P_i/NaCl)BSA$ , even at the highest concentration tested, did not compete with either AGE-BSA or  $(P_i/NaCl)BSA$ , presumably because the level of AGE in  $(P_i/NaCl)BSA$  is so low. Yeast mannan was also ineffective

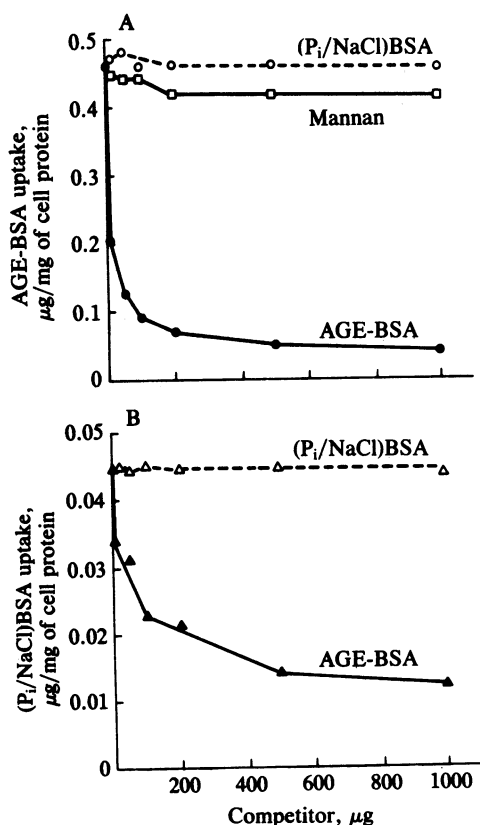


FIG. 2. Mouse macrophage uptake of <sup>125</sup>I-labeled AGE-BSA (A) and <sup>125</sup>I-labeled  $(P_i/NaCl)BSA$  (B) in the presence of various concentrations of unlabeled competitors. Each well contained 10 µg of <sup>125</sup>I-labeled protein and the indicated amount of AGE-BSA (● in A; ▲ in B),  $(P_i/NaCl)BSA$  (○ in A; △ in B), or mannan (□). After incubation for 4 hr at 37°C, the amount of <sup>125</sup>I-labeled material in the cells was determined in duplicate.

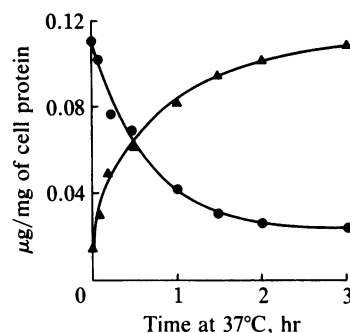


FIG. 3. Degradation at 37°C of <sup>125</sup>I-labeled AGE-BSA previously bound at 4°C by mouse macrophages. After incubation of the cells with <sup>125</sup>I-labeled AGE-BSA (10 µg/ml) at 4°C for 2 hr, the radioligand-containing medium was removed and each monolayer was washed five times with ice-cold medium. Each well then received 1 ml of medium at 37°C and incubation was continued at 37°C. At intervals, both the amount of radiolabeled material remaining associated with the cells (●) and the amount of trichloroacetic acid-soluble <sup>125</sup>I-labeled material released into the medium (▲) were determined in duplicate.

as an AGE-BSA competitor, indicating that AGE-BSA recognition is not associated with the mannose/fucose receptor (17).

Preliminary binding experiments with constant concentrations of labeled AGE-BSA at 4°C indicated that a 2-hr incubation period was necessary for maximal binding. When cells that had been incubated with <sup>125</sup>I-labeled AGE-BSA (10 µg/ml) at 4°C for 2 hr were warmed to 37°C, cell-bound radioactivity declined rapidly, while trichloroacetic acid-soluble radioactivity in the medium increased concomitantly (Fig. 3). Postbinding endocytosis and degradation was nearly complete at 60 min.

When macrophages were incubated at 4°C with increasing concentrations of AGE-BSA, specific binding increased in a saturable fashion (Fig. 4A). Assuming that each labeled AGE-BSA molecule has one ligand recognizable by the receptor, maximal specific binding of <sup>125</sup>I-labeled AGE-BSA to cell monolayers ranged between 1.2 and 1.4 pmol/mg of cell protein; 50% saturation of binding sites was reached at 30–40 nM. Scatchard analysis of these data (Fig. 4B) indicated that there are  $\approx 1.06 \times 10^5$  receptors per macrophage with an affinity constant of  $1.75 \times 10^{-11}$  M. High-affinity binding of <sup>125</sup>I-labeled AGE-BSA at 4°C was reduced >85%

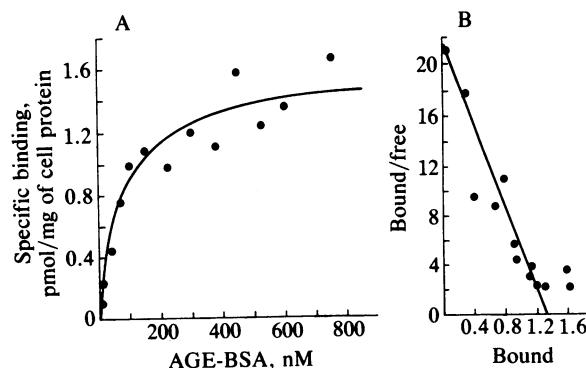


FIG. 4. (A) Specific binding of <sup>125</sup>I-labeled AGE-BSA to mouse peritoneal macrophages. The saturation-binding profile was obtained by incubating macrophages with various concentrations of <sup>125</sup>I-labeled AGE-BSA at 4°C for 2 hr. Nonspecific binding was determined in the presence of a 100-fold excess of unlabeled AGE-BSA. Specific binding was determined on duplicate samples as described in *Methods*. (B) Scatchard analysis of binding data in A. Units for the Scatchard plot are as follows: Bound, pmol/mg of cell protein; Bound/free, pmol-mg<sup>-1</sup>·nM<sup>-1</sup>.

when the cells were preincubated with 0.05% trypsin for 30 min at 37°C. In contrast, cells treated with trypsin after a 4-hr incubation with labeled AGE-BSA at 37°C retained >80% of the cell-associated radioactivity.

Specific binding of AGE-BSA to its macrophage receptor was competitively inhibited by BSA coupled to FFI. As indicated in Fig. 5, FFI-BSA suppressed AGE-BSA binding to ≈25% of control (100% value was 0.460 μg/mg of cell protein). Cellular uptake of <sup>125</sup>I-labeled FFI-BSA at 37°C, like AGE-BSA uptake, increased with concentration in a saturable fashion (Fig. 6). However, only 30% as much protein accumulated in the cells at similar concentrations. This suggests that the AGE receptor may have a higher affinity for other AGE than it does for FFI.

<sup>125</sup>I-labeled FFI-BSA uptake was inhibited to <35% of the control value by a 100-fold excess of unlabeled AGE-BSA (data not shown), confirming that these substances are taken up via the same binding site. <sup>125</sup>I-labeled (P<sub>i</sub>/NaCl)BSA did not compete with FFI-BSA. In addition, the yeast polysaccharide mannan had no effect on the uptake of <sup>125</sup>I-labeled FFI-BSA (data not shown). To define further the nature of the receptor-ligand interaction, excess unlabeled FFI-hexanoic acid alone, unattached to protein, was incubated with cells in the presence of <sup>125</sup>I-labeled AGE-BSA (1 μg/ml). However, trypan blue exclusion showed that these concentrations of FFI were toxic to the cells (unpublished data), and competition data were thus unobtainable.

Prior incubation of macrophages with unlabeled AGE-BSA (25 μg/ml or 250 μg/ml) for 72 hr did not diminish the rate at which the macrophages took up and degraded <sup>125</sup>I-labeled AGE-BSA, indicating that the AGE receptor is not down-regulated by its ligand (data not shown).

## DISCUSSION

The studies presented here demonstrate the presence of a specific high-affinity receptor on mouse macrophages that binds BSA that has been modified by long-term exposure to glucose (AGE-BSA). Binding of AGE-BSA is followed by rapid intracellular accumulation and degradation. Yeast mannan does not compete with AGE-BSA uptake, showing that the AGE receptor is unrelated to the mannose/fucose receptor. The number of AGE receptors per cell is similar to that reported for the macrophage Fc receptor, but the AGE-receptor binding affinity is substantially higher (18). Binding

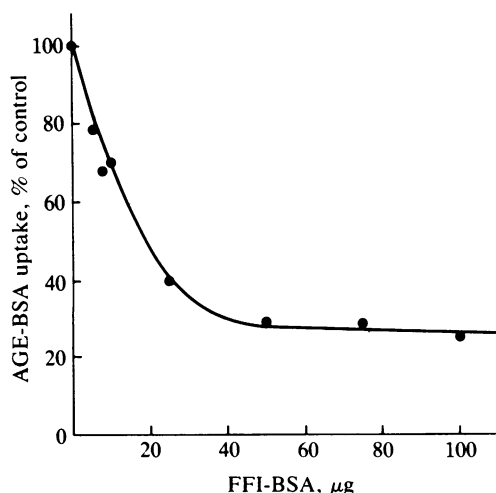


FIG. 5. Competitive inhibition of <sup>125</sup>I-labeled AGE-BSA (10 μg/ml) uptake by FFI-BSA. Incubation was at 37°C for 4 hr. The intracellular content of <sup>125</sup>I-labeled AGE-BSA was determined in triplicate.

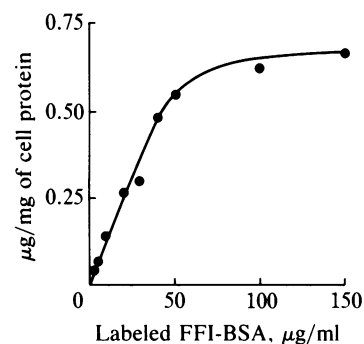


FIG. 6. Macrophage accumulation of <sup>125</sup>I-labeled FFI-BSA. After a 4-hr incubation at 37°C in 1.0 ml of medium containing labeled FFI-BSA, cellular uptake was determined in triplicate.

data used to calculate the AGE-receptor number and affinity constant in these studies were expressed in terms of pmol of <sup>125</sup>I-labeled AGE-BSA bound. Since each molecule of BSA may contain more than one AGE recognizable by the receptor, however, the results obtained by Scatchard analysis must be regarded as probable underestimations. The AGE receptor does not appear to be regulated by exposure to AGE-proteins, since prior incubation of macrophages with AGE-BSA failed to influence the subsequent uptake and degradation of labeled AGE-BSA. Small amounts of (P<sub>i</sub>/NaCl)BSA are also recognized by this receptor. Uptake of (P<sub>i</sub>/NaCl)BSA is competitively inhibited by the addition of excess unlabeled AGE-BSA, whereas excess (P<sub>i</sub>/NaCl)BSA has no effect. This most likely reflects the presence, in preparations of (P<sub>i</sub>/NaCl)BSA, of small amounts of preexisting AGE-BSA formed *in vivo*. Small amounts of FFI have in fact been detected by RIA in freshly isolated albumin (10).

Receptor-mediated AGE-BSA uptake is also competitively inhibited by BSA with chemically attached FFI. This suggests that the AGE receptor recognizes a specific type of AGE structure having important homology with FFI. Competition experiments using unattached FFI-hexanoic acid were attempted in order to further substantiate this point, but the compound at these concentrations was found to be cytotoxic (unpublished data). Thus, it is not certain that high-affinity binding of AGE-proteins requires the specific furoyl-furanyl-imidazole structure. *In vivo*, a whole family of AGE form on proteins during long-term exposure to glucose (19, 20), and a number of these may bind to the AGE receptor with specific individual affinities.

Although we used AGE-BSA as a model protein to characterize the AGE receptor, relatively little AGE accumulates on such short-lived proteins at glucose concentrations found *in vivo* (8, 10). Rather, AGE accumulates predominantly on proteins that turn over at a much slower rate, such as collagen, elastin, and myelin (8). AGE-myelin formation *in vivo* as well as *in vitro* has also been shown to result in specific recognition and uptake by macrophages. AGE-myelin uptake is inhibitable by both AGE-myelin and AGE-BSA but not by (P<sub>i</sub>/NaCl)myelin or (P<sub>i</sub>/NaCl)BSA (5).

These observations indicate that AGE-receptor-mediated endocytosis occurs with modified proteins formed *in vivo* and suggest that other proteins accumulating significant amounts of AGE *in vivo* will be similarly recognized and degraded by macrophages. The efficiency of this removal system is not complete, however, since a linear increase in highly cross-linked AGE-collagen accumulation occurs with age (2–4). This age-related increase in AGE-collagen reduces elasticity in arteries, heart, and lungs. Reduced elasticity, in turn, contributes to continuous decline in such physiological functions as cardiac index, renal blood flow, vital capacity, and maximum oxygen uptake (21). The inability of macrophages to completely remove senescent macromolecules may thus

be responsible, in part, for development of physiologically important age-related changes. Increased understanding of AGE-receptor-mediated endocytosis may therefore provide further insight into the regulatory mechanisms of aging.

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1. Pinnell, S. P. (1978) in *The Metabolic Basis of Inherited Disease*, eds. Stanbury, J. B., Wyngaarden, J. B. & Fredrickson, D. S. (McGraw-Hill, New York), p. 1366.
2. Schnider, S. L. & Kohn, R. R. (1980) *J. Clin. Invest.* **66**, 1179–1181.
3. Schnider, S. L. & Kohn, R. R. (1981) *J. Clin. Invest.* **67**, 1630–1635.
4. Monnier, V. M., Kohn, R. R. & Cerami, A. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 583–587.
5. Vlassara, H., Brownlee, M. & Cerami, A. (1984) *J. Exp. Med.* **160**, 197–207.
6. Koenig, R. J. & Cerami, A. (1980) *Annu. Rev. Med.* **31**, 29–34.
7. Mortensen, H. B. & Christophersen, C. (1983) *Clin. Chim. Acta* **134**, 317–326.
8. Brownlee, M., Vlassara, H. & Cerami, A. (1984) *Ann. Int. Med.* **101**, 527–537.
9. Pongor, S., Ulrich, P. C., Bencsath, F. A. & Cerami, A. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2684–2688.
10. Chang, J. C. F., Ulrich, P. C., Bucala, R. & Cerami, A. (1985) *J. Biol. Chem.*, in press.
11. Vlassara, H., Brownlee, M. & Cerami, A. (1985) *Diabetes* **34**, 553–557.
12. Vlassara, H., Brownlee, M. & Cerami, A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5190–5192.
13. Edelson, P. J. & Cohn, Z. A. (1976) in *In Vitro Methods in Cell Mediated and Tumor Immunity*, eds. Bloom, B. R. & David, J. R. (Academic, New York), pp. 333–340.
14. Fraker, P. J. & Speck, J. C. (1978) *Biochem. Biophys. Res. Commun.* **80**, 849–855.
15. Bradford, M. (1976) *Anal. Biochem.* **72**, 248–254.
16. Scatchard, G. (1949) *Ann. N. Y. Acad. Sci.* **51**, 660–672.
17. Imber, M. J., Pizzo, S. V., Johnson, W. J. & Adams, D. O. (1982) *J. Biol. Chem.* **257**, 5129–5135.
18. Unkeless, J. C. (1977) *J. Exp. Med.* **145**, 931–947.
19. Reynolds, T. M. (1963) *Adv. Food Res.* **12**, 1–52.
20. Reynolds, T. M. (1965) *Adv. Food Res.* **14**, 167–283.
21. Shock, N. W. (1980) in *Aging—Its Chemistry*, ed. Dietz, A. A. (Am. Assoc. Clin. Chem., Washington, DC), p. 3.